

ABSTRACT

DNA-PK ASSAY

The present invention provides a method for detecting DNA-activated protein kinase (DNA-PK) activity in a biological sample. The method includes contacting a biological sample with a detectably-labeled phosphate donor and a synthetic peptide substrate defined by the following features to provide specific recognition and phosphorylation by DNA-PK: (1) a phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and, (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor. A composition and a kit for the detection of DNA-PK activity are also provided. Methods for detecting

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20 DNA, protein phosphatases and substances that alter the activity of DNA-PK are also provided. The present invention also provides a method of monitoring protein kinase and DNA-PK activity in living cells. A composition and a kit for monitoring protein kinase activity *in vitro* and a composition and a kit for monitoring DNA-PK activities in living cells are also provided. A method for identifying agents that alter protein kinase activity *in vitro* and a method for identifying agents that alter DNA-PK activity in living cells are also provided.

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